

78. A method for preparing a bioactive protein comprising at least one cysteine bridge comprising:

expressing a recombinant protein comprising a first peptidyl fragment, a second peptidyl fragment comprising an amino acid sequence which comprises at least two cysteine residues which form at least one cysteine bridge in a bioactive conformation of the second peptidyl fragment, and at least one cleavable peptidyl fragment linking the first and second peptidyl fragments, where the first peptidyl fragment mediates formation of the bioactive conformation of the second peptidyl fragment;

causing the second peptidyl fragment to adopt the bioactive conformation.

79. A method according to claim 78 wherein causing the second peptidyl fragment to adopt the bioactive conformation includes contacting the recombination protein with an aqueous medium comprising at least one chaotropic auxiliary agent.

80. A method according to claim 78 wherein the at least one chaotropic auxiliary agent is urea.

81. A method according to claim 79 wherein the urea is present in a concentration between about 2 M to 8 M.

82. A method according to claim 81 wherein the urea is present in a concentration between about 3 M to 6 M.

83. A method according to claim 78 wherein the aqueous medium further comprises a mercaptan.

84. A method according to claim 83 wherein the mercaptan is selected from the group consisting of dithiothreitol, dithioerythrol, 2-mercaptoethanol, cysteine, methyl thioglycolate, 3-mercapto-1,2-propanediol and 3-mercaptopropionic acid.

85. A method according to claim 83 wherein the mercaptan is 2-mercaptoethanol.

86. A method according to claim 79 wherein the aqueous medium has a pH between about 8 and 10.5.
87. A method according to claim 79 wherein the aqueous medium has a pH between about 9 and 10.
88. A method according to claim 79 wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.05 and 15 grams per liter.
89. A method according to claim 79 wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.5 and 5 grams per liter.
90. A method according to claim 79 wherein the recombinant protein is present in the medium in a concentration between about 2 and 3 grams per liter.
91. A method according to claim 78 wherein causing the second peptidyl fragment to adopt the bioactive conformation includes contacting the recombinant protein with a mercaptan.
92. A method according to claim 91 wherein the mercaptan yields less than 5 -SH radical of the mercaptan per cysteine residue of recombinant protein.
93. A method according to claim 91 wherein sufficient mercaptan is provided to yield between about 0.07 to about 1.0 -SH radical of the mercaptan per cysteine residue of recombinant protein.
94. A method according to claim 78, further comprising isolating a portion of the expressed recombinant protein which is in the bioactive conformation.
95. A method according to claim 94 wherein isolating is performed by ultrafiltration.
96. A method according to claim 95 wherein ultrafiltration is performed at a pH between about 8 and 11.

97. A method according to claim 95 wherein ultrafiltration is performed at a pH between about 9 and 10.

98. A method according to claim 78 wherein the second peptidyl fragment exhibits insulin-like bioactivity in its bioactive conformation.

99. A method according to claim 78 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

100. A method according to claim 78 wherein the second peptidyl fragment is an insulin precursor.

101. A method according to claim 78 wherein the second peptidyl fragment is an insulin precursor of human origin.

102. A method according to claim 78 wherein the second peptidyl fragment comprises SEQ. ID. No. 4.

103. A method according to claim 78 wherein the second peptidyl fragment comprises SEQ. ID. No. 5.

104. A method according to claim 78 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

105. A method according to claim 78 wherein the second peptidyl fragment comprises at least four cysteine residues which form two cysteine bridges.

106. A method according to claim 78 wherein the second peptidyl fragment comprises at least six cysteine residues which form three cysteine bridges

107. A method according to claim 106 wherein the first peptidyl fragment is capable

of being bound by an anti-hGH antibody.

108. A method according to claim 107 wherein the first peptidyl fragment comprises SEQ. ID. No. 1.

109. A method according to claim 107 wherein the first peptidyl fragment comprises SEQ. ID. No. 2.

110. A method according to claim 107 wherein the first peptidyl fragment comprises SEQ. ID. No. 3.

111. A method according to claim 107 wherein the first peptidyl fragment is between 20 and 200 residues in length.

112. A method according to claim 78 wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

113. A method according to claim 78 wherein the first peptidyl fragment comprises SEQ. ID. No. 1.

114. A method according to claim 78 wherein the first peptidyl fragment comprises SEQ. ID. No. 2.

115. A method according to claim 78 wherein the first peptidyl fragment comprises SEQ. ID. No. 3.

116. A method according to claim 78 wherein the first peptidyl fragment is between 20 and 200 residues in length.

117. A method according to claim 78 wherein the C-terminus of the first peptidyl fragment is adjacent the N-terminus of the second peptidyl fragment.

118. A method according to claim 78 wherein the N-terminus of the first peptidyl

fragment is adjacent the C-terminus of the second peptidyl fragment.

119. A method according to claim 78 wherein the first peptidyl fragment is positioned within the second peptidyl fragment.

120. A method according to claim 78 wherein the method further includes cleaving the at least one cleavable peptidyl fragment.

121. A method according to claim 78 wherein the at least one cleavable peptidyl fragment is an Arg or Lys residue.

122. A method according to claim 78 wherein the at least one cleavable peptidyl fragment is at least 2 amino acids in length where the C-terminal amino acid residue is selected from the group consisting of Arg and Lys.

123. A chimeric protein comprising:
a first peptidyl fragment;
a second peptidyl fragment comprising an amino acid sequence which exhibits insulin-like bioactivity when folded in a bioactive conformation; and
at least one cleavable peptidyl fragment linking the first and second peptidyl fragments;
wherein the first peptidyl fragment is selected such that it mediates folding of the second peptidyl fragment to cause the second peptidyl fragment to adopt the bioactive conformation.

124. A protein according to claim 123 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

125. A protein according to claim 123 wherein the second peptidyl fragment is an insulin precursor.

126. A protein according to claim 123 wherein the second peptidyl fragment is an insulin precursor of human origin.

127. A protein according to claim 123 wherein the second peptidyl fragment comprises SEQ. ID. No. 4.

128. A protein according to claim 123 wherein the second peptidyl fragment comprises SEQ. ID. No. 5.

129. A protein according to claim 123 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

130. An assay for improving bioactive conformation mediation activity comprising:
taking an amino acid sequence of a first recombinant protein comprising a first peptidyl fragment, a second peptidyl fragment comprising an amino acid sequence which comprises at least two cysteine residues which form at least one cysteine bridge in a bioactive conformation of the second peptidyl fragment, and a cleavable peptidyl fragment linking the first and second peptidyl fragments, where the first peptidyl fragment has sufficient homology to at least a first 20 N-terminal amino acids of human growth hormone (hGH) protein that the first peptidyl fragment mediates formation of the bioactive conformation of the second peptidyl fragment;

expressing a second recombinant protein where the amino acid sequence of the first peptidyl fragment has been modified relative to the first recombinant protein; causing the second peptidyl fragment of the second recombinant protein to adopt the bioactive conformation;

determining a yield for the step of causing the second peptidyl fragment of the second recombinant protein to adopt the bioactive conformation; and

comparing the yield for the step of causing the second peptidyl fragment of the second recombinant protein to adopt the bioactive conformation to a yield for causing the second peptidyl fragment of the first recombinant protein to adopt the bioactive conformation.